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Morphological variation and chromosome studies in *Calligonum mongolicum* and *C. pumilum* (Polygonaceae) suggests the presence of only one species

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Calligonum spp. are common plant species in desert areas of central Asia. Since they are drought-resistant, salt-tolerant and grow successfully in sand, they are useful for wind-breaks and dune stabilization. The fruit morphology is regarded as a key taxonomic character for the genus. Morphological variation among individuals and populations of *Calligonum mongolicum* Turcz. and *C. pumilum* Losinsk. is investigated in the present paper. The chromosome number for both species is also presented and found to be the same ($2n = 18$). By combining the overlap and variation in morphological characters, the similar variational ranges and complex relationships of fruit characters, we suggest that *C. pumilum* should be merged with *C. mongolicum* and we further discuss additions and improvements to the description of *C. mongolicum* in 'Flora of China'.

Calligonum L. species are shrubs and sub-shrubs distributed in Asia, southern Europe and northern Africa, with desert species found in Middle Asia (Mao and Pan 1986). The morphological and anatomical characters of the fruits are regarded as key characters in *Calligonum* taxonomy (Mao and Pan 1986). The taxonomic history of *Calligonum* is one of flux (Kang and Zhang 2007). Losinskaja (1927) published 10 new species: *C. gobicum, C. chinense*, *C. potaninii*, *C. pumilum, C. roborowskii*, *C. alashanicum*, *C. zaidamense*, *C. kozlovi*, *C. potaninii* and *C. klementzii.* In succession, Pavlov (1936) listed 117 species of *Calligonum* in the world. Then Soskov (1975b) treated *C. gobicum, C. chinense*, *C. pumilum, C. roborowskii*, *C. alashanicum*, and *C. zaidamense* as synonyms of *C. mongolicum.* Mao (1984) later published *C. trifarium*, *C. kengisaricum*, *C. korlaense* and *C. jeminaicum* and in 1985 Liu published *C. juochiangense*. Botanists in China currently recognize 38 species and 11 varieties of *Calligonum* in the world, with 23 species in China (Bao and Grabovskaya-Borodina 2003). Natural hybrids often produce variable phenotypes adding to the taxonomic confusion in the genus (Mao and Pan 1986). As a whole, the section *Medusa*, which contains *C. mongolicum* and *C. pumilum,* is considered taxonomically problematic.

Calligonum mongolicum is the earliest named species in section *Medusa* (Mao and Pan 1986), and it has the widest geographic distribution of all *Calligonum* species, ranging from Xinlinhaote of Neimeng in China on the east to the Hami and Tushantuo Basin in Xinjiang in western China. The species reaches the south of Luobupo along the Sule Debouchment and Milan in the east of Rouqiang in China and reaches also Daban City which is in the west of Neimenggu, Beita Mountain, Qitai and the east of Urumqi in Xinjiang. The geographic distribution of *C. pumilum* is smaller and within that of *C. mongolicum*, restricted to Yiwu, Hami and Shansan in the east of Xinjiang (Mao and Pan 1986).

Calligonum pumilum is similar to *C. mongolicum* in both morphology and geographic distribution. *Calligonum pumilum* was first named by Losinskaja (1927). Because of the fruit characters, Soskov (1975a) later included *Calligonum pumilum* in *C. mongolicum.* In contrast, Mao (1984) maintained *C. pumilum* when she edited 'Flora of China', because she took the number of rows of bristles on each rib (NRR) as a key character to distinguish the two species.

'Flora Xinjiangensis' states that *C. pumilum* "... is similar to *C. mongolicum*, but the achenes of the former have one row of bristles on each rib, thus it is easy to distinguish them" (Yang et al. 1992). Thus, from comparison of the characters described in 'Flora of China' (Table 1), we know that *C. mongolicum* and *C. pumilum* are morphologically similar, except for the number of rows of bristles on each rib (NRR) and plant height (H). However, in our initial investigation of these two species, we found that NRR

Table 1. The taxonomic characters of *Calligonum mongolicum* and *C. pumilum* as given in 'Flora of China'.

Character	*C. mongolicum*	*C. pumilum*
Habit	shrub	shrub
Plant height (cm)	25–150	30–50
Old branch color	grayish white or light yellow–gray	gray or light yellow–gray
Current year branch color	gray–green	gray–green
Internode distance (cm)	0.6–3.0	1.0–3.5
Leaf length (mm)	2–4	2–3
Flower number per axil	2 or 3	2 or 3
Flower position	at leaf axil	at leaf axil
Fruit form	broadly ellipsoid	broadly ellipsoid
Fruit size (mm)	8–12 ×7–11	7–12 ×6–8
Number of rows of bristles on each rib	2 or 3	1
Flower phenology	May–Jul	Apr–May
Fruit phenology	Jun–Aug	May–Sep
Chromosome number	2n =18, (27)	2n =18

is not a consistent character, and plant height did not appear to be correlated to the NRR, especially not in saline habitats in Erlianhaote, Neimenggu, China, where we found some very small *C. mongolicum* which were only 2–5 cm tall but had 2 or 3 rows of bristles in each rib. Given these initial findings, the aim of the present study was to perform a survey of the morphology of wild populations, discern useful taxonomic characters, and clarify the taxonomic status of these two important *Calligonum* species.

Material and methods

From 31 July to 31 August 2006 we investigated wild populations of *C. mongolicum* and *C. pumilum* in Wuhai, Dengkou and Alasamen of Neimenggu in the east of the Xinjiang Province. We noted the longitude, latitude and altitude, and collected specimens. We identified specimens based on their external morphology and distinguished populations on whether or not the fruits had one row of bristles (P) on each rib or not (M). Vouchers are preserved at XJBI. Collection data for both species is presented in Table 2.

We selected 10 individual plants in each population. The roots of *Calligonum* are able to produce ramets, so we kept a 40 m distance between individuals during sampling in order to avoid sampling the same individual twice. We also measured the canopy area (CA) and height of each plant (H). We selected 10 fruits from each plant, and noted the number of bristle rows on each rib (NRR). We used calipers to measure the length of the fruit (LF), width of fruit (WF), the length of setae (LS), the distance between setae (SS), the distance between ribs (SR), the length of achenes (LA) and the width of achenes (WA), and to calculate the fruit form (FF = LF/WF) and the achene form (AF = LA/WA) (Tao et al. 2001). We used a general chromosome squash method to count the chromosome number (Li and Song 1997).

Data processing

We used the Kruskal–Wallis test to analyze differences among individuals within the populations, as well as differences among the populations of both species. We also used the Mann–Whitney U-test to analyze differences between *C. mongolicum* and *C. pumilum* in fruit characters, canopy area (CA) and heights of plants (H). We used Excel 2007 and SPSS ver. 15.0 for the statistical analyses (Tao and Ren 2004).

Results

The results in Fig. 1 indicate that NRR is variable and overlapping in *C. mongolicum* and *C. pumilum*. There are two types of NRR occurring in M1, M2, P1 and P3, which is inconsistent at the among-population level. Two morphotypes (2 NRR and 3 NRR) were observed *C. mongolicum* which is consistent with the description in 'Flora of

Table 2. Origin of the analyzed populations of *Calligonum mongolicum* and *C. pumilum.*

Taxon	Population	Location	Appraiser	Specimen number	Collection date	Habitat	Climate
C. mongolicum	M1	Erlianhaote, Neimeng, China 112°03′E, 43°45′N, alt. 898 m	Borong Pan	06005–06014	10 Aug 2006	sand dunes	arid desert
	M2	Qingtongxia, Ninxia, China 105°55′E, 38°01′E, alt. 1134 m	Borong Pan	06020–06029	19 Aug 2006	gravel gobi	continental semi-arid
	M3	Erjinaqi, China 100°26′E, 41°27′N, alt. 1002 m	Borong Pan	06056–06067	24 Aug 2006	sand dunes	extremely arid desert
C. pumilum	P1	Hami, Xinjiang, China 091°32′E, 43°23′N, alt.1038 m	Borong Pan	05010–05019	9 Jul 2005	gravel gobi	continental temperate zone
	P2	Hami, Xinjiang, China 091°23′E, 43°20′N, alt.1273 m	Borong Pan	06198–06207	30 Aug 2006	desert varnish	continental temperate zone
	P3	Liuyuan, Gansu, China 095°28′E, 95°28′N, alt.1744 m	Borong Pan	06163–06172	29 Aug 2006	gravel gobi	landlocked arid

Figure 1. Comparison of the number of bristle rows on each rib in different populations of *Calligonum mongolicum* (M1–M3) and *C. Pumilum* (P1–P3).

China', but two types (1 NRR and 2 NRR) were also found in populations of *C. pumilum* which is inconsistent with 'Flora of China' (Bao and Grabovskaya-Borodina 2003).

We selected 10 individual plants in each population of *C. mongolicum* and *C. pumilum* and measured their canopy (CA) and height (H), as shown in Table 3.

The results in Table 3 show that the minimum canopy area (CA) of plants in population M1, M2 and M3 is 0.010 m^2, the maximum is 14.004 m^2, minimum height is 2.1 cm, and the maximum is 120.2 cm. According to the description in 'Flora of China', the plant height of *C. mongolicum* is ca 25–150 cm. Compared to M1, M2 and M3, the differences among P1, P2 and P3 are smaller; the minimum of CA is 0.645 m^2, the maximum is 10.337 m^2, the minimum height is 33.2 cm, the maximum is 149.8 cm.

The statistical results show that there were significant differences (Kruskal–Wallis $H = 0.000–0.005$, $p < 0.05$) in CA and H among populations of both species, but at the species-level the CA of *C. mongolicum* and *C. pumilum* were similar (Mann–Whitney $U = 0.264$, $p > 0.05$), while the heights were significantly different (Mann–Whitney $U = 0.000$, $p < 0.05$). Because of the observed overlap and variation in canopy area (CA) and heights (H) of *C. mongolicum* and *C. pumilum* these traits are not taxonomically reliable and may not be used to distinguish these two species.

The result presented in Fig. 2 shows that the variation observed in nine fruit characters do not represent significant differences between *C. mongolicum* (M1, M2 and M3) and *C. pumilum* (P1, P2 and P3). The variational ranges of two fruit characters of P3 (LF: 0.585 ~ 1.432 cm; WF: 0.236 ~ 0.562 cm) were similar to M1 (LF: 0.588 ~ 1.000 cm; WF: 0.226 ~ 0.652 cm), and two of P3 (FF: 0.771 ~ 4.102; AF: 1.255 ~ 4.700) were similar to M3 (FF: 0.569 ~ 3.286; AF: 1.308 ~ 4.640), and the ranges of variation of three fruit characters of P1 (LS: 0.104 ~ 0.248 cm; LA: 0.500 ~ 0.860 cm; AF: 1.780 ~ 3.943) were similar to those of M1 (LS: 0.102 ~ 0.502 cm; LA: 0.500 ~ 0.966 cm; AF: 1.255 ~ 4.700). The variational ranges of SS, SR and WA in six populations were similar too, as shown in Fig. 2.

All of the indexes except FF (fruit form) in M1 (Kruskal–Wallis $H = 0.130$, $p > 0.05$) and SS (space between setae) (Kruskal–Wallis $H = 0.566$, $p > 0.05$) in P1 were significantly different (Kruskal–Wallis $H = 0.000–0.016$, $p < 0.05$) between plants. The fruit indexes (LF, WF, LS, SS, SR, LA, WA, FF and AF) were significantly different among the species within the same population. The fruit characters were significantly different

Table 3. Canopy area (CA) and plant height (H1) in three populations each of *Calligonum mongolicum* (M1, M2, M3) and *C. pumilum* (P1, P2, P3). Avg = average.

	M1		M2		M3	
	CA (m^2)	H (cm)	CA (m^2)	H (cm)	CA (m^2)	H (cm)
C. mongolicum						
Avg	0.036	3.2	2.064	105.7	7.362	76.4
Min	0.010	2.1	1.128	80.3	2.380	52.0
Max	0.089	5.5	4.097	120.2	14.004	109.2
	P1		P2		P3	
	CA (m^2)	H (cm)	CA (m^2)	H (cm)	CA (m^2)	H (cm)
C. pumilum						
Avg	6.403	82.5	2.198	64.4	2.641	113.3
Min	2.800	41.4	0.645	33.2	1.537	70.3
Max	10.337	101.6	3.666	82.1	5.149	149.8

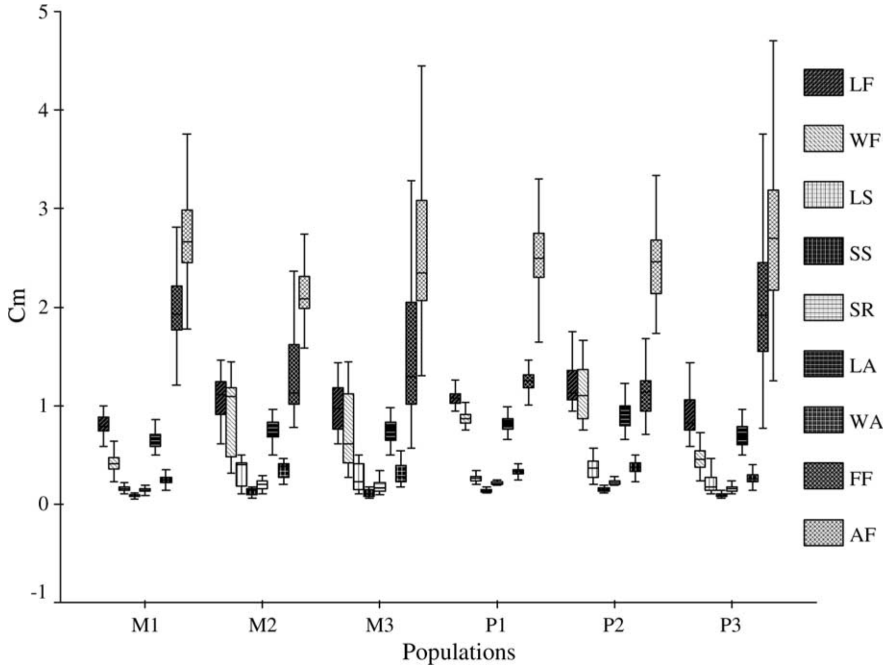

Figure 2. The variation of nine fruit characters in different populations. NRR = number of bristle rows in each rib, LF = length of fruit, WF = width of fruit, LS = length of setae, SS = space between setae, SR = space between ribs, LA = length of achenes, WA = width of achenes, FF = fruit form (FF = LF/WF), AF = achene form (AF = LA/WA).

(Kruskal–Wallis H = 0.000, $p < 0.05$) among M1, M2 and M3, and also among P1, P2 and P3 (Kruskal–Wallis H = 0.000–0.011, $p < 0.05$). This indicates that the differences in fruit characters vary significantly among the populations of the same. Of nine fruit characters in *C. mongolicum* and *C. pumilum*, seven characters (LF, WF, LS, SS, SR, WA, FF) were significantly different (Mann–Whitney U = 0.000–0.017, $p < 0.05$) between the two species; only two (viz LA and AF) were not significant (Mann–Whitney U = 0.190 and 0.369, $p < 0.05$).

The somatic chromosome number of *C. mongolicum* and *C. pumilum* were both found to be $2n = 18$ (Fig. 3), as has previously been reported by Wang and Guan (1986). However, these authors also reported $3n = 27$ for *C. mongolicum*.

Discussion

The NRR has been the key taxonomic index to distinguish *C. mongolicum* from *C. pumilum*. We found two types

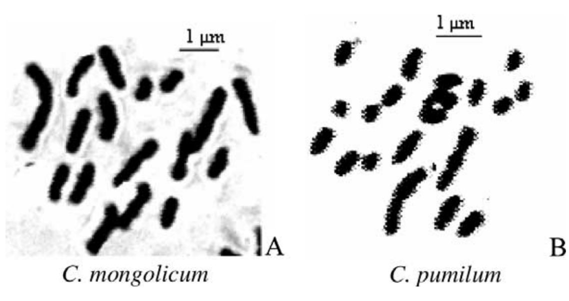

Figure 3. Chromosomes of *Calligonum mongolicum* and *C. pumilum*.

NRR in populations of *C. mongolicum* (2 NRR and 3NRR) and two in *C. pumilum* (1 NRR and 2 NRR), so we can not say that NRR is unambiguous for each species. A similar result has been reported concerning the morphological fruit characteristics of different populations in *C. ebi-nuricum* (Kang et al. 2008).

Calligonum mongolicum or *C. pumilum* have overlaping geographic distributions; the intraspecific variation observed may be preserved through the vegetative propagation and perennial habit.

The individual plants within the same population of *C. mongolicum* or *C. pumilum* (except the AF of M1 and the SS of P1, $p > 0.05$) all showed significant differences in fruit morphology. This is consistent with the morphological variation among populations of *C. rubicundum* (Tao and Ren 2004). This indicates that fruit characters between individuals in the same population are usually significantly different. These significant morphological differences among individuals in the same population may be caused by genetic variability, micro-scale adaptation or environmentally-induced differences (phenotypic plasticity). There was no significant difference for AF in M1 among the individuals, probably because their fruits are smaller than normal for the species, and the same phenomenon appeared in the SS in P1 is because of their close setae.

We observed significant differences in fruit characters among the populations of the same species of *C. mongolicum* or *C.pumilum*. There are three possible causes for this observation: 1) phenotypic plasticity, which can play a large role in plant morphology (Ren and Tao 2004) – how much environmental resources (light, temperature, water, etc.) among populations in nature is required to cause a large difference in morphology is not known but should be considered as a possible explanation (Ren and Tao 2004); 2) local selection (assuming restricted gene flow among populations and high selection coefficients); or 3) genetic drift (assuming restricted gene flow, small effective population size and selectively neutral morphological phenotypes) (Tremblay 1997, Van Rossum et al. 1997). Taking into account the large and significant morphological differences observed among individuals in populations of both *C. mongolicum* or *C. pumilum*, we do not see any possibility for the continued separation of *C. pumilum* from *C. mongolicum* based on fruit characters.

The results show that the differences of fruit characters between populations of *C. mongolicum* and *C. pumilum* are significant, which supports the classical taxonomic characters as reasonable. But when combining the significant differences within the same population and the significant differences among the populations of both species, the fruit characters can not be considered taxonomically reliable for distinguishing *C. mongolicum* from *C. pumilum*. Therefore, we support the opinion of Soskov that these two species are better treated as one variable species.

The somatic chromosome number of both *C. mongolicum* and *C. pumilum* were found to be $2n = 18$. The same chromosome number for *C. mongolicum* and *C. pumilum* indicates a similar evolutionary origin. Provided that the morphological variance of *C. mongolicum* is not dominated by phenotypic plasticity, the present results suggest that genetic drift and founder events may cause much of the variance in morphological traits among populations, perhaps because of low gene flow and small population size.

In conclusion, because of the overlap and variation in the characters NRR, CA and H, the similar variation ranges and the complex relationship among fruit characters, and the same chromosome number ($2n = 18$), we suggest that *Calligonum pumilum* should be merged with *C. mongolicum*.

Morphological description of *Calligonum mongolicum* s. l. (including *C. pumilum*)

Shrubs 2–150 cm tall. Old branches spreading, flexuous, grayish white or pale yellow–gray; herbaceous branches of current year gray-green, jointed; joints 0.6–3.5 cm. Leaves linear, 2–4 mm; ocrea united with the leaf. Pedicel 1–2 mm, slender, jointed below middle. Flowers 2 or 3, at leaf axil, white or pale red. Tepals spreading in fruit, ovate, ca 2 mm. Fruit broadly ellipsoid, 0.5–1.2 cm $\times$ 2–12 mm. Achenes linear, narrowly ellipsoid, or broadly ellipsoid, not to very coiled; ribs prominent or not, each with 1, 2 or 3 rows of bristles; bristles dense, as long as or slightly longer than the width of the achenes, slender, breakable, not or slightly enlarged at base, 2- or 3-branched from middle. Flowering: Apr, May, Jul. Fruiting: May, Jun, Aug, Sep. $2n = 18$, (27).

Mobile, semi-mobile, or stable sand dunes, deserts; 500–1800 m a.s.l. Gansu, Nei Mongol, Ninxia Xinjiang (Mongolia).

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